OVULATION AND IMPLANTATION IN *MICROTUS* AS A FUNCTION OF COPULATORY BEHAVIOR

By

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OVULATION AND IMPLANTATION IN MICROTUS AS A FUNCTION OF COPULATORY BEHAVIOR

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This research is concerned with the effects of variations in male copulatory behavior on ovulation and implantation in three species of *Microtus*, *M. pennsylvanicus*, *M. montanus*, and *M. ochrogaster*. Variations in both the amount of copulatory behavior and the temporal and frequency pattern of copulatory behavior were investigated. The first of four experiments examined the amount of copulatory behavior necessary to induce ovulation and implantation in estrous *M. pennsylvanicus* females. A single ejaculatory series with a high number of intromissions (12-29) produced ovulation and implantation in 70% of the females tested, whereas a single ejaculatory series with only a few intromissions (1-4) was insufficient. Additional series produced a slight increase in the probability of ovulation and implantation and significant increases in the numbers of corpora lutea and implanted embryos. The second experiment evaluated the copulatory behavior pattern of *M. pennsylvanicus* males mated with *M. pennsylvanicus*, *M. montanus*, and *M. ochrogaster* females. Inter-specific mating did not result in any major alteration in the temporal and frequency pattern of copulatory behavior for *M. pennsylvanicus* males. The third experiment examined the effect of variations in the pattern of copulatory behavior on ovulation and implantation in *M. ochrogaster* and *M. montanus* females. *M. pennsylvanicus* males perform their species-typical pattern of copulatory behavior in inter-specific matings, a pattern clearly distinct from the copulatory behavior pattern of *M. ochrogaster* and *M. montanus* males. Thus, the effectiveness of *M. pennsylvanicus* males in inducing ovulation and implantation in *M. ochrogaster* and *M. montanus* females was compared to the effectiveness of the females' conspecific males. *M. pennsylvanicus* males were as effective as *M. ochrogaster* males in inducing ovulation in *M. ochrogaster* females. *M. pennsylvanicus* males were not as effective as *M. montanus* males in inducing ovulation in *M. montanus* females. Only 50% of the females ovulated with *M. pennsylvanicus* males compared to 100% with *M. montanus* males. Inter-specific matings did not result in any implanted embryos. The fourth experiment examined the failure of implantation in the inter-specific matings of Experiment 3. Two days following copulation, ova were examined in *M. ochrogaster* and *M. montanus* females mated with *M. pennsylvanicus* or conspecific males. Inter-specific mating did not result in development of ova into a two cell stage. Results of the experiments were discussed with respect to the adaptive significance of the elaborateness and diversity of copulatory behavior.

INTRODUCTION

Copulatory behavior in mammals typically is an elaborate behavior pattern, involving a complex series of motor patterns and requiring a considerable expenditure of time and energy (Dewsbury, 1972). For example, in the laboratory rat, the male performs a series of discrete mounts, on some of which he briefly inserts the penis into the vagina and then dismounts. Approximately ten of these mounts with insertion (intromissions) precede the occurrence of a mount with insertion and ejaculation. The series concludes with an ejaculation. Males typically show approximately seven ejaculatory series before reaching a satiety criterion of 30 min with no intromission, and copulation may continue for several hours. Copulatory behavior tends to be highly stereotyped within species, but shows substantial diversity among different species (Dewsbury, 1972). Male rats always perform several pre-ejaculatory intromissions, each with one pelvic thrust, before ejaculation. Male guinea pigs, on the other hand, regularly perform no pre-ejaculatory intromissions but evidence several pelvic thrusts on a single ejaculatory intromission. A similar pattern of copulation occurs in humans. Male dogs demonstrate a lock or mechanical connection between male and female during copulation. Recent research has provided a series of systematic, quantitative descriptions of copulatory behavior in a number of species from the rodent family Cricetidae. The descriptions reveal substantial diversity even within this one family. Certain species have locks (Dewsbury and Jansen, 1972; Dewsbury, 1974), others require multiple intromissions to ejaculation (Dewsbury, 1970; 1971; 1973), and one species can ejaculate on a single intromission with several pelvic thrusts (Gray and Dewsbury, 1974).

The function, or adaptive significance, of the two basic characteristics of copulatory behavior, elaborateness and diversity, is not well understood. What is the utility of all the behavior involved in copulation? Why is there such diversity in the pattern of copulation, even within one family? Investigation of the functions of copulatory behavior focuses on the consequences of the behavior in relation to such factors as habitat, social organization, ecology, reproductive anatomy, and reproductive physiology. The present research concerns the consequences of copulatory behavior for female reproductive physiology.

Copulatory Behavior and Female Reproductive Physiology

There are ample data which suggest that copulatory behavior functions to modulate female reproductive physiology. It is clear that one function of copulatory behavior is the transfer of sperm from male to female. This relationship, and its importance to female reproduction, has been recognized for thousands of years, and it is the attribute common to all patterns of copulatory behavior. Yet, ejaculation in most species occupies only a small percentage of the time and energy spent in copulation. Therefore, the function of sperm transfer can not account for either the elaborateness of copulatory behavior or the diversity of the behavior patterns.

For many species, the stimulation provided by copulatory behavior can have important effects on various components of female reproductive physiology. Conaway (1971) has proposed a classification schema for female reproductive systems based on their dependence on copulation. Two components of female reproduction are the focus of this classification, ovulation and implantation. In one type of system, typified by such species as cats and rabbits, ovulation is "induced" or dependent on the occurrence of copulation. The development of functional corpora lutea (CL) and an extended luteal phase of progesterone secretion in the ovarian cycle follows ovulation automatically. The functional CL secrete large amounts of progesterone which acts to prepare the uterine wall for implantation of the embryo. In a second type of system, typified by laboratory rats and mice, ovulation is "spontaneous" or independent of the occurrence of copulation. Implantation, however, is dependent on copulation; functional CL and preparation of the uterus for implantation are not evidenced unless copulatory stimulation has been received. In a third type of system, typified by primates, ovulation is spontaneous, and the development of functional CL and preparation of the uterus for implantation follows ovulation automatically. Thus, both ovulation and implantation are independent of copulation.

Three additional facts emphasize the importance of copulatory stimulation for female reproduction, even for species which show spontaneous ovulation and implantation. First, accumulation of data on a wide variety of species indicates that induced ovulation may be a more widespread phenomenon than spontaneous ovulation. Induced ovulation also may be the phylogenetically older form, and presumably, spontaneous ovulation evolved from an induced ovulation system (Conaway, 1971). Second, there is clear evidence of effects of copulation on ovulation in a number of species which are commonly viewed as spontaneous ovulators (Jöchle, 1973). In laboratory rats, for example, copulation can elicit ovulation in estrogen-primed, diestrous females (Aron, Asch, and Roos, 1966); in females where spontaneous ovulation has been blocked experimentally with barbiturates (Zarrow and Clark, 1968), constant light (Dempsey and Searles, 1943; Brown-Grant, Davidson, and Greig, 1973), or perinatal androgenization (Ericsson and Baker, 1966); and in females in constant or persistant estrus (Everett, 1939). Normal, cycling females show an increase in the number of ova ovulated following copulation as compared to unmated females (Rodgers, 1971). In house mice, induced ovulation has been demonstrated under certain conditions (Zarrow, Campbell, and Clark, 1968). The timing of ovulation has been altered by copulation in pigs (Signoret, du Mesnil du Buisson, and Mauleon, 1972) and sheep (Van der Wesherysen, van Niekerk, and Hunter, 1970). Finally, Jöchle (1973) has provided data on human females which suggest a correlation between copulatory stimulation and the occurrence of ovulation. Third, in addition to ovulation and implantation, several other components of female reproduction can be affected by copulation. These include sperm transport (Adler, 1969; Chester and Zucker, 1970; Sullivan, Bartlett, Elliot, Brouwer, and Klock, 1972), mammary development (Dilley and Adler, 1968), and parturition (Diamond, 1972). In conclusion then, for many if not all species, a second function of copulatory behavior is to provide stimulation necessary to elicit responses of the female reproductive system which maximize successful reproduction. The adaptive significance of both the elaborateness and diversity of copulatory behavior may thus be related to stimulation requirements of the female reproductive systems.

The amount of copulatory stimulation is a critical variable in the stimulation requirements of some species. In laboratory rats, several pre-ejaculatory intromissions are necessary for the induction of luteal activity, sperm transport, and ultimately successful pregnancy (Wilson, Adler, and LeBoeuf, 1966; Adler, 1969). Moreover, an increasing number of intromissions (1-16) results in an increasing probability of cessation of behavioral receptivity, suggesting a cumulative effect of copulatory stimulation on luteal activity (Adler, 1969). Ball (1934) provided data which indicate an increased probability of luteal activity in female rats receiving two ejaculatory series as compared to females receiving only a single series. A number of other species also require multiple series for maximal responsiveness of the female's reproductive system. Hamster females require more than four ejaculatory series to insure a high probability of luteal activity and successful pregnancy (Lanier, Estep, and Dewsbury, 1974). Female montane voles (Microtus montanus), which are induced ovulators, require more than two ejaculatory series to insure a high probability of ovulation (Davis, Gray, Zerylnick, and Dewsbury, 1974). Pearson (1944) has reported that short-tailed shrews require at least 19 locks with ejaculation to induce ovulation. Other species require no more than one ejaculatory series. Prairie voles (Microtus ochrogaster) require one ejaculatory series to insure a high probability of ovulation. A number of pre-ejaculatory intromissions and intravaginal thrusts must be included in the one series however (Gray, Zerylnick, Davis, and Dewsbury, 1974). House mice have been reported to respond to the occurrence of ejaculation itself (McGill and Coughlin, 1971; McGill, 1972) although other data suggest a possible role for the pre-ejaculatory intromissions and thrusts (Diamond, 1970). Alpacas require only one or two intromissions to induce ovulation (Fernandez-Boca, Madden, and Novoa, 1970). In general, the requirement of females for copulatory stimulation, in many instances a substantial amount of stimulation, would seem to provide some explanation for the elaborateness of male copulatory behavior. At present however, data are limited to only a few species.

It has been proposed that the pattern of copulatory stimulation is a critical variable in the stimulation requirements of females (Diamond, 1970). In this view, females of a given species are maximally responsive not simply to specific amounts of copulatory stimulation but to the temporal and frequency pattern of stimulation provided by conspecific males. Diamond (1970) has proposed the term "vaginal code" to describe this specificity to a particular pattern of stimulation. Using a mechanical vibrator to provide stimulation to induce luteal activity, Diamond and Yanagimachi (1968) reported that female hamsters are more responsive to a number of discrete insertions (5 sec each) than to a single insertion (150 sec). The most successful procedure for inducing luteal activity was to stimulate females 30 times (5 sec each) over 300 sec; fewer insertions, or lengthening or shortening the exposure duration markedly decreased success. Evidence also suggests a possible "vaginal code" in house mice (Diamond, 1970). This proposal of "vaginal codes" would explain, to some extent, the highly stereotyped diversity in copulatory behavior patterns. The data, however, are not clear-cut on the existence of vaginal codes, and research has been limited to a few laboratory species. The present study concerns the effects of copulatory behavior on female reproductive physiology in three species of voles (*Microtus*).

Microtus Reproductive Biology

The three *Microtus* species employed in the present research, *M. pennsylvanicus*, *M. montanus*, and *M. ochrogaster*, offer a number of advantages for examining questions of function for copulatory behavior. The animals are small rodents which are adaptable to the laboratory (Lee and Horvath, 1969; Richmond and Conaway, 1969b). They breed exceptionally well and are easily observed under experimental conditions. Yet, they have not undergone the many generations of artificial selection as have the typical laboratory species. The three species are native to North America. *M. pennsylvanicus*, or the meadow vole, is found throughout the northern United States and Canada; *M. ochrogaster*, or the prairie vole, occurs in the midwestern United States; and *M. montanus*, or the montane vole, is found in the area of the Rocky Mountains (Hall and Kelson, 1959). The ranges of the species overlap to some extent. *M. pennsylvanicus* is sympatric with *M. ochrogaster* in areas of the Midwest and with *M. montanus* in areas of the Rocky Mountains.

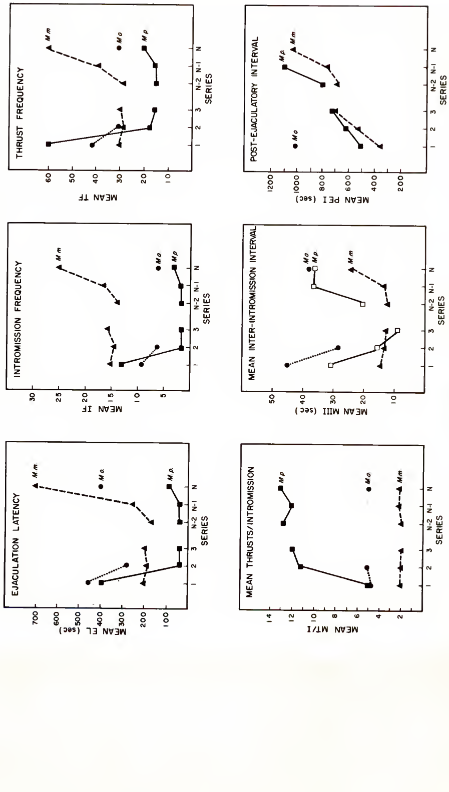
There are considerable data on the reproductive physiology of the species. Females of each species show induced ovulation (Richmond and Conaway, 1969a; Clulow and Mallory, 1970; Lee, Horvath, Metcalfe, and Inskeep, 1970; Gray, Davis, Zerylnick, and Dewsbury, 1974). Females do not have regular estrous cycles but acylic, extended periods of estrus, diestrus, and occasionally proestrus (Richmond and Conaway, 1969a; Clulow and Mallory, 1970; Gray *et al.*, 1974). The age of sexual maturity has been estimated from 35-60 days for males and females of the three species (Seidel and Booth, 1960; Lee and Horvath, 1969;

Richmond and Conaway, 1969b). Finally, *Microtus* are prolific breeders in the natural habitat, and they have been studied extensively because of their dramatic population fluctuations (Christian and Davis, 1964; Krebs, Keller, and Tamarin, 1969).

Copulatory behavior in the three species of *Microtus* clearly evidences the two general characteristics of mammalian copulation, elaborateness and diversity. Quantitative data are available on the behavior of each species (Dewsbury, 1973; Gray and Dewsbury, 1973, 1974). Each species performs a substantial amount of behavior during copulation, with a number of pre-ejaculatory intromissions and thrusts in each series (Figure 1). Multiple series occur in all three species, ranging from a mean of 2.0 for *M. ochrogaster* to means of 5.0 and 5.9 for *M. montanus* and *M. pennsylvanicus*, respectively. Each species also performs a highly stereotyped pattern of copulatory behavior which is distinct from the other two species. Differences among the species involve quantitative aspects of the behavior and the pattern of change across series (Figure 1). The rate of thrusting during intromission also varies among the species. *M. ochrogaster* males have a thrust rate of approximately one thrust every 2 sec while *M. pennsylvanicus* and *M. montanus* show a substantially faster rate of one thrust per sec. It has been proposed that these highly stereotyped differences in the pattern of copulatory behavior are related to differences in the stimulation requirements of the females and that these differences serve as a reproductive isolating mechanism (Gray and Dewsbury, 1973). According to this hypothesis, females possess a vaginal code, and events in their reproductive system are triggered by a distinct pattern of copulatory behavior. The differences

FIGURE 1

Mean scores on selected quantitative measures of copulatory behavior for *M. montanus* (Dewsbury, 1973), *M. ochrogaster* (Gray and Dewsbury, 1973), and *M. pennsylvanicus* (Gray and Dewsbury, 1974). Measures are defined in text (see pages 22-23). Data are illustrated for the initial three series and last three series, where appropriate (N refers to the last complete series before attainment of the satiety criterion).


EJACULATION LATENCY
MEAN EL (sec)
700
600
500
400
300
200
100
1
2
3
N-2
N-1
N
SERIES
Mm
Mo
Mp
INTROMISSION FREQUENCY
MEAN IF
30
25
20
15
10
5
1
2
3
N-2
N-1
N
SERIES
Mm
Mo
Mp
THRUST FREQUENCY
MEAN TF
60
50
40
30
20
10
1
2
3
N-2
N-1
N
SERIES
Mm
Mo
Mp
MEAN THRUSTS/INTROMISSION
MEAN MT/I
14
12
10
8
6
4
2
1
2
3
N-2
N-1
N
SERIES
Mp
Mo
Mm
MEAN INTER-INTROMISSION INTERVAL
MEAN MIII (sec)
50
40
30
20
10
1
2
3
N-2
N-1
N
SERIES
Mo
Mp
Mm
POST-EJACULATORY INTERVAL
MEAN PEI (sec)
1200
1000
800
600
400
200
1
2
3
N-2
N-1
N
SERIES
Mo
Mp
Mm

in pattern among the *Microtus* species would allow females to respond only to mating by a conspecific male. Males of a sympatric species would not be effective in eliciting responses of the female's reproductive system, and hence, efficient reproductive isolation of the species would be achieved. As stated above, *M. pennsylvanicus* occurs sympatrically with both *M. montanus* and *M. ochrogaster*. In both instances of sympatry, there is evidence of social interactions between individuals of the different species (Koplin and Hoffmann, 1968; Krebs *et al.*, 1969), but no hybrids have been reported (Gray, 1970). The reproductive isolating mechanisms among the species have not been identified (Miller, 1969).

Data are already available for two of the species on the stimulation requirements of females with respect to amounts of copulatory stimulation (Davis *et al.*, 1974; Gray *et al.*, 1974). Comparison of these requirements with the copulatory behavior for conspecific males reveals a correlation between male copulatory behavior and female stimulation requirements (Gray *et al.*, 1974). *M. montanus* females require more than two ejaculatory series to insure a high probability of ovulation and implantation, and *M. montanus* males are able to attain a mean of 5.0 series before sexual satiety. On the other hand, *M. ochrogaster* females require only one series, and *M. ochrogaster* males rarely attain more than 2.0 series before sexual satiety. Within the one ejaculatory series, females require approximately 8 pre-ejaculatory intromissions, and males perform a mean of 9.5 intromissions before ejaculation on the first series. This apparent coadaptation of male copulatory behavior and female stimulation requirements provides substantial evidence for the importance of female stimulation requirements in explaining the elaborateness of male copulatory behavior.

The present research is designed to further examine the effects of copulatory behavior on ovulation and implantation in Microtus. Examination of female stimulation requirements in M. pennsylvanicus will provide important information on the validity of the correlation between male copulatory behavior and female stimulation requirements with respect to amounts of copulatory stimulation. There are no data in Microtus on the stimulation requirements of females with respect to patterns of copulatory stimulation. Given the distinct patterns of copulation in Microtus, investigation of patterning effects will provide important data on the relevance of female stimulation requirements in explaining the diversity of male copulatory behavior patterns. The investigation will also serve to test the hypothesis of reproductive isolation as a function for the distinct patterns of copulation in Microtus.

EXPERIMENT 1

The species-typical pattern of copulatory behavior in M. pennsylvanicus involves several ejaculatory series, with each series containing a number of pre-ejaculatory intromissions and intravaginal thrusts (Figure 1). Experiment 1 concerns the amount of copulatory stimulation necessary to induce ovulation and implantation in M. pennsylvanicus females.

Methods

Subjects

Subjects were 40 adult female M. pennsylvanicus (age 4-6 mos). Thirteen adult male M. pennsylvanicus (age 4-12 mos) were used as mating partners, and several additional adult males were employed as stimulus males for the induction of estrus in females (see below). Animals were obtained from a colony maintained over several generations at the University of Florida. All animals were of proven fertility (i.e., each had produced at least one litter). Animals were housed individually in clear, plastic cages, 48 x 27 x 13 cm for males and 29 x 19 x 13 cm for females. "Purina Rabbit Chow" and water were available at all times, with fresh lettuce supplied once a week. Animals were maintained on a reversed 16:8 light-dark cycle (lights on 8:00 p.m. - 12:00 noon).

Procedures

Females were brought into estrus by being placed in close proximity to adult males (Richmond and Conaway, 1969a). Plastic cages (48 x 27 x 13 cm) were divided in half by removable hardware cloth barriers, with a female housed on one side and a stimulus male on the other. The reproductive state of the females was evaluated by daily vaginal smears. This technique involved collection of epithelial cells from the vaginal wall with a wire loop, staining the cells with toluidine blue, and examining the cells under a microscope. In *Microtus*, as in many rodent species, changes in the types of cells lining the vaginal wall reliably reflect changes in the reproductive state of the female. Vaginal smears were initiated three days prior to placement of the females into the divided cage in order to accustom the females to the procedure. Mating tests were conducted on the second consecutive day of estrus following placement into the divided cage. Estrus was defined by 50% or more cornified cells in the vaginal smear.

Mating tests were conducted 2-4 hr following light offset. Tests were initiated by placing an estrous female into the home cage of the male partner. If the female was judged unreceptive or the male failed to achieve an intromission within 30 min, the test was discontinued and discounted. Females were judged unreceptive if they failed to show a lordotic response after several vigorous mounting attempts by the male.

Mating tests involved one of four experimental conditions. In the first condition (n=10), the male was removed from the cage before the female was introduced, and thus no copulation was permitted. In the second condition (n=10), the male and female were permitted to copulate through one ejaculatory series following introduction of the female; the male was then removed from the cage. In the third condition (n=10), the male and female also were permitted to copulate through one ejaculatory series before removal of the male partner. However, before introduction of the experimental female, the male partner was allowed one ejaculatory series with a lure female. Thus, the ejaculatory series received by the experimental female was the second for the male. The distinction between the male's first and second series is important when one considers the amount of copulatory behavior contained in each series (see Figure 1). Females mated on a single second series (third condition) should receive substantially less stimulation than females mated on a single first series (second condition). In the fourth condition (n=10), the male and female were observed through two ejaculatory series and then left to copulate for an additional 2 hr; the male was then removed from the cage. Although normative data indicate that this amount of time should allow copulation to satiety (defined as 30 min with no intromission), three males were observed continuously for partial confirmation. No male partner was employed more than once in any condition. In all conditions, the females remained in the home cage of the male partner following mating until sacrificed.

Females were killed eight days following the mating tests. Their ovaries and uteri were examined under a dissecting microscope. The number of CL was recorded for each ovary. Since each CL develops from an ovulated follicle, the number of CL should accurately reflect the number of ova released. The number of implanted embryos was recorded for each uterine horn, and a measure of intra-uterine mortality was then calculated by subtracting the number of implanted embryos from the number of CL. Microscopic examination of several serially sectioned ovaries was conducted in order to verify the accuracy of the CL counts. Ovaries were fixed in Bouin's solution, embedded in paraffin, sectioned at 10 u, and stained with hematoxylin and eosin.

Results

The percentage of females ovulating (showing at least one CL) in each of the four experimental conditions is shown in Table 1. Unmated females failed to ovulate. Among mated females, the percentage of females ovulating increased as a function of increasing amounts of copulatory stimulation. None of the females receiving a single second series ovulated, 70% of the females receiving a single first series ovulated, and 100% of the females mated to satiety ovulated.

Quantitative data on ovulation and implantation were collected for the two experimental conditions in which ovulation occurred (Table 1). Among females which ovulated, those mated to satiety showed a significantly greater number of CL and implanted embryos as compared to females receiving a single first series. There was no difference in intrauterine mortality. Histological examination of the ovaries revealed no discrepancies with CL counts obtained with the dissecting microscope.

The three pairs observed continuously in the satiety condition all reached satiety and showed a mean of 5.0 series (range 4-6). A mean of 2.0 intromissions (range 1-4) and 20.0 thrusts (range 14-24) occurred on the second series; a mean of 18.7 intromissions (range 12-29) and 66.4 thrusts (49-94) occurred on the first series.

TABLE 1

EFFECTS OF VARYING AMOUNTS OF COPULATORY STIMULATION ON OVULATION AND IMPLANTATION IN *M. PENNSYLVANICUS* FEMALES

Measure	Copulatory Stimulation				P [a]
	Unmated	One Ejac. Series (second)	One Ejac. Series (first)	Satiety	
Number of females	10	10	10	10	—
Percentage of females ovulating	0	0	70	100	<.01 (X^2=31.41)
Mean number of corpora lutea [b]	—	—	4.57 ± 0.48	6.10 ± 0.41	<.05 (t=2.42)
Mean number of implanted embryos [b]	—	—	3.71 ± 0.29	4.90 ± 0.23	<.01 (t=3.23)
Mean intra-uterine mortality [b, c]	—	—	0.86 ± 0.26	1.20 ± 0.36	NS

[a] Results of statistical analyses.

[b] Only females which ovulated are included in the results.

[c] Number of CL minus number of implanted embryos.

Discussion

Results indicate that M. pennsylvanicus females require more than one ejaculatory series to insure maximal ovulation and implantation. Additional copulation following the first ejaculation results in a greater probability of ovulation and a maximal number of CL and implanted embryos. This effect of multiple series indicates an important function for the pattern of multiple series in M. pennsylvanicus males. The significance of multiple series in M. pennsylvanicus might also be that they permit males to inseminate more than one female. However, this is unlikely since ejaculatory series subsequent to the first contain only a few intromissions and thrusts and do not provide sufficient stimulation to the female to induce ovulation and implantation.

Recent research indicates that multiple series are necessary for successful reproduction in several rodent species, including montane voles (Davis et al., 1974), hamsters (Lanier et al., 1974), and cactus mice (Dewsbury and Estep, 1974). The adaptive value of a female reproductive system relatively unresponsive to a single series might be to increase overall fitness of the female's litter either through selection of a vigorous male or through repeated insemination. For species in which females typically mate with just one male, the requirement of multiple ejaculations might insure that ova are released only after matings with healthy and vigorous males (assuming that the ability to achieve multiple ejaculations is positively correlated with health and vigor). For species in which females mate with several different males, the requirement might insure sperm competition and genetic diversity of the litter. Insemination of one female by several males has been reported in the field for at least one muroid rodent (Birdsall and Nash, 1973).

The increase in the number of CL and implanted embryos with multiple series presumably is due to an increase in the number of ova released. Research on the two other species of *Microtus*, *M. montanus* and *M. ochrogaster*, did not find any effect of varying amounts of copulation on the number of CL or implanted embryos although there was a clear effect on the probability of ovulation (Davis *et al.*, 1974; Gray *et al.*, 1974). The lack of an effect in *M. montanus* and *M. ochrogaster* may have been due to greater individual variability as a result of the greater age range in the females. Preliminary data on *M. pennsylvanicus* in our laboratory suggest that age may substantially alter the sensitivity of the female's reproductive system to copulatory stimulation.

M. pennsylvanicus is only the second species in which amount of copulation has been demonstrated to effect the number of ova ovulated. In laboratory rats, Aron, Asch, and Roos (1966) have reported an increase in CL number as a function of increased numbers of intromissions (coits) in estrogen-primed, diestrous females; and Rodgers (1971) has demonstrated an increase in the number of ova released in mated, proestrous females as compared to unmated females. The mechanism of this effect on ova number, as well as the effect of increased probability of ovulation itself, presumably involves the neuroendocrine regulation of LH release from the pituitary. A substantial increase in plasma levels of LH follows mating in proestrous female rats, and there are data to suggest a functional relationship between amounts of copulation and LH levels (Moss and Cooper, 1973). An increase in plasma LH also follows mating in rabbits (Dufy-Barbe, Franchimont, and Faure, 1973) and field voles, Microtus agrestis (Charlton, Naftolin, and Worth, 1969). Research is necessary to evaluate the relationship of LH and copulatory behavior in Microtus, and work in our laboratory is in progress on the validation of a radioimmunoassay for LH in Microtus.

EXPERIMENT 2

As mentioned previously, it has been proposed that a major function of species differences in copulatory behavior among *Microtus* is reproductive isolation (Gray and Dewsbury, 1973). An assumption underlying this hypothesis is that the male largely determines the pattern of copulation and hence the temporal and frequency characteristics of the copulatory stimulation. For example, *M. pennsylvanicus* males should perform their species-typical pattern of copulatory behavior whether mating with *M. pennsylvanicus* females or females of another species. At present, there are no quantitative data on copulatory behavior in inter-specific matings. Experiment 2 concerns the copulatory behavior of *M. pennsylvanicus* males mating with *M. montanus*, *M. ochrogaster*, and *M. pennsylvanicus* females.

Methods

Subjects

Normative data on copulatory behavior were collected on 15 male *M. pennsylvanicus* (age 6-12 mos). Females used as mating partners included 10 female *M. pennsylvanicus* (age 4-6 mos), 10 female *M. ochrogaster* (age 6-12 mos), and 10 female *M. montanus* (age 5-12 mos). Several *M. pennsylvanicus*, *M. ochrogaster*, and *M. montanus* males were used as stimulus males for the induction of estrus in conspecific females. All animals were obtained from colonies maintained over several generations at the University of Florida. Each animal was of proven fertility.

Procedures

The testing procedure was as described in Experiment 1.

M. pennsylvanicus males were mated with: 1) M. pennsylvanicus females, 2) M. montanus females, 3) M. ochrogaster females. Pairs were observed through three ejaculatory series, and the behavior was recorded on an Esterline-Angus event recorder. Ten males were tested in each condition, with 10 M. pennsylvanicus, 10 M. montanus, and 10 M. ochrogaster females employed as mating partners. Seven males participated in all three experimental conditions, and tests were spaced at least 10 days apart.

The copulatory behavior of the males was evaluated using several standard, quantitative measures (Dewsbury, 1973 ; Gray and Dewsbury, 1973; 1974). In these measures, the behavior of the male is divided into ejaculatory series, with each series containing an ejaculation and all copulatory events leading up to the ejaculation. The measures describe the temporal and frequency characteristics of the three basic motor patterns of copulation in M. pennsylvanicus: mounts (without vaginal penetration), intromissions (with vaginal penetration and intravaginal thrusting), and ejaculations (Gray and Dewsbury, 1974). The temporal measures include:

Intromission Latency (IL) - latency (in sec) from introduction of the female to the first intromission.

Ejaculation Latency (EL) - latency (in sec) from the first intromission of a series to the ejaculation.

Post-Ejaculatory Interval (PEI) - latency (in sec) from the occurrence of an ejaculation to the next, subsequent intromission.

The frequency measures include:

Intromission Frequency (IF) - total number of intromissions in a series including the ejaculatory intromission.

Thrust Frequency (TF) - total number of intravaginal thrusts in a series.

Mean Thrusts per Intromission (T/I) - mean number of thrusts per intromission in a series, obtained by dividing TF by IF.

Mount Frequency (MF) - total number of mounts without vaginal penetration in a series.

Results

M. pennsylvanicus males showed the same pattern of copulatory behavior whether mated with *M. pennsylvanicus*, *M. montanus*, or *M. ochrogaster* females (Table 2). Of the 18 quantitative measures of copulatory behavior, only Intromission Latency was significantly different across the three females. Observation of the animals indicated that this difference in IL occurred as a result of substantial species differences in the females' initial response to the male. *M. pennsylvanicus* and *M. montanus* females were much more aggressive during the initial approaches of the male, thereby prolonging the time required by the male to mount the female. The basic motor patterns of copulation were also unchanged across the three females.

Discussion

The pattern of copulatory behavior and the temporal and frequency characteristics of the copulatory stimulation are largely a function of the male. The role of the female is limited, at least as reflected by the quantitative measures. It is possible, then, that female

TABLE 2

EFFECTS OF INTER-SPECIFIC MATING ON THE
COPULATORY BEHAVIOR OF _M. PENNSYLVANICUS_ MALES

Measure		Females			F	P
		M. penn.	_M. ochro._	_M. mont._		
IL	Mean	557.0	374.0	805.4	4.92	<.05
	S.E.	108.76	96.82	82.69		
IF-1 [a]	Mean	18.7	17.5	19.5	1.90	NS
	S.E.	1.83	1.92	2.51		
TF-1	Mean	65.6	66.8	69.6	0.13	NS
	S.E.	4.35	5.08	5.09		
T/I-1	Mean	3.2	3.9	3.7	3.07	NS
	S.E.	0.13	0.26	0.20		
EL-1	Mean	599.6	510.0	650.8	0.83	NS
	S.E.	84.74	78.33	72.09		
MF-1	Mean	27.6	30.8	27.7	0.36	NS
	S.E.	2.93	3.13	3.00		
PEI-1	Mean	571.2	583.5	553.5	0.12	NS
	S.E.	35.07	54.88	37.17		
IF-2	Mean	2.0	2.1	2.5	1.68	NS
	S.E.	0.33	0.46	0.43		
TF-2	Mean	20.0	19.0	21.8	0.55	NS
	S.E.	1.37	2.16	2.12		
T/I-2	Mean	11.8	11.1	10.2	0.41	NS
	S.E.	1.29	1.20	1.07		
EL-2	Mean	38.2	53.7	32.6	1.01	NS
	S.E.	5.57	16.29	7.61		

[a] Number following hyphen identifies series.

TABLE 2 (continued)

Measure		Females			F	P
		M. penn.	*M. ochro.*	*M. mont.*		
MF-2	Mean	2.3	2.0	2.1	0.18	NS
	S.E.	0.50	0.47	0.49		
PEI-2	Mean	637.5	760.9	707.0	2.41	NS
	S.E.	38.35	50.43	37.60		
IF-3	Mean	2.0	1.9	1.7	0.23	NS
	S.E.	0.33	0.39	0.26		
TF-3	Mean	19.4	19.6	19.0	0.02	NS
	S.E.	2.02	2.17	2.0		
T/I-3	Mean	11.3	11.5	12.0	0.38	NS
	S.E.	0.95	0.82	0.71		
EL-3	Mean	29.5	34.4	23.9	1.79	NS
	S.E.	3.02	5.33	2.95		
MF-3	Mean	2.1	2.5	1.5	1.04	NS
	S.E.	0.53	0.52	0.36		

Microtus can differentially respond to males of different species on the basis of copulatory stimulation. To examine this possibility, Experiment 3 was conducted.

EXPERIMENT 3

It has been suggested that the pattern of copulatory stimulation is a critical variable in the stimulation requirements of *Microtus* females. It also has been suggested that the existence of vaginal codes with respect to patterns of stimulation functions to maintain reproductive isolation between sympatric *Microtus* species. *M. pennsylvanicus* males perform their species-typical pattern of copulatory behavior in inter-specific matings with *M. ochrogaster* and *M. montanus* females. Moreover, the copulatory pattern of *M. pennsylvanicus* is clearly distinct from the pattern of *M. ochrogaster* and *M. montanus*. Experiment 3 provides a test of the above proposals by examining the effectiveness of copulation by *M. pennsylvanicus* males in inducing ovulation and implantation in *M. ochrogaster* and *M. montanus* females.

Methods

Subjects

Subjects were 20 female *M. ochrogaster* (age 4-12 mos) and 20 female *M. montanus* (age 4-6 mos). Fourteen male *M. pennsylvanicus* (6-12 mos), 10 male *M. ochrogaster* (age 6-12 mos), and 10 male *M. montanus* (age 5-10 mos) were employed as mating partners. Several additional *M. ochrogaster* and *M. montanus* males from the colonies were used as stimulus males for the induction of estrus in conspecific females. All animals were of proven fertility.

Procedures

The testing procedure was as described in Experiment 1.

Mating tests for *M. ochrogaster* females involved one of two experimental conditions: 1) copulation with a *M. pennsylvanicus* male, 2) copulation with a *M. ochrogaster* male. In each condition, the male and female were observed through three ejaculatory series and then left to copulate for an additional 2 hr; the male was then removed from the cage. Three pairs in each condition were observed continuously in order to confirm that copulation continued to satiety. Ten females were tested in each condition, with 10 *M. pennsylvanicus* and 10 *M. ochrogaster* males employed as mating partners.

Mating tests for *M. montanus* females involved either: 1) copulation with a *M. pennsylvanicus* male, 2) copulation with a *M. montanus* male. The male and female were observed through three ejaculatory series and then allowed to continue copulation for an additional 2 hr; the male was subsequently removed from the cage. However, three pairs from each condition were observed continuously. Ten females were tested in each condition, with 10 *M. pennsylvanicus* and 10 *M. ochrogaster* males employed as mating partners.

Females were killed eight days following mating. The number of CL and implanted embryos were recorded for each female, and a measure of intra-uterine mortality was calculated. Several ovaries from each experimental condition were prepared for histological examination as described in Experiment 1.

Results

M. pennsylvanicus males were as effective as *M. ochrogaster* males in inducing ovulation in *M. ochrogaster* females (Table 3). All females ovulated whether mated with *M. pennsylvanicus* or *M. ochrogaster* males, and there were no differences in the appearance of CL or in the number of CL. Histological examination of the ovaries confirmed these findings. None of the females mated with *M. pennsylvanicus* males had implanted embryos whereas all females mated with *M. ochrogaster* males showed at least one implanted embryo. Observation of six pairs throughout the mating tests revealed that all males mated to satiety, with a mean of 5.3 series (range 5-6) for *M. pennsylvanicus* males and 2.3 series (range 2-3) for *M. ochrogaster* males.

M. pennsylvanicus males were not as effective as *M. montanus* males in inducing ovulation in *M. montanus* females (Table 4). Only 50% of the females mated with *M. pennsylvanicus* males ovulated while 100% of the females mated with *M. montanus* males ovulated. Given the occurrence of ovulation, there were no differences between the two experimental conditions in the appearance of CL or the number of CL. These findings were confirmed by histological examination of several ovaries. None of the females mated with *M. pennsylvanicus* males had implanted embryos; all of the females mated with *M. montanus* males had implanted embryos. All of the males observed throughout the mating tests reached satiety, with a mean of 5.6 series (range 5-7) for *M. pennsylvanicus* males and 4.6 series (4-5) for *M. montanus* males.

Discussion

Results of the experiment indicate that the pattern of copulatory

TABLE 3

EFFECT OF MATING BY *M. PENNSYLVANICUS* AND *M. OCHROGASTER* MALES ON OVULATION IN *M. OCHROGASTER* FEMALES

	Males	
Measure	*M. ochro.*	*M. penn.*
Number of females	10	10
Percentage of females ovulating	100	100
Mean number of corpora lutea [a]	4.5 ± 0.34	4.4 ± 0.33

[a] Only females which ovulated are included in the results.

TABLE 4

EFFECT OF MATING BY *M. PENNSYLVANICUS* AND *M. MONTANUS* MALES ON OVULATION IN *M. MONTANUS* FEMALES

Measure	Males		P [a]
	M. mont.	*M. penn.*	
Number of females	10	10	—
Percentage of females ovulating	100	50	$<.01$ ($X^2=6.66$)
Mean number of corpora lutea [b]	5.6 ± 0.37	5.6 ± 0.51	NS ($t=0.00$)

[a] Results of statistical analyses.

[b] Only females which ovulated are included in the results.

stimulation is not a critical variable in the stimulation requirements of M. ochrogaster and M. montanus females. M. ochrogaster females clearly show maximal ovulation in response to mating by M. pennsylvanicus males even though the pattern of copulation in M. pennsylvanicus males differs substantially from the pattern in M. ochrogaster males. The results for M. montanus females are not as straightforward. M. montanus females do not ovulate reliably following mating by M. pennsylvanicus males. This failure of ovulation may be due to the differences between M. pennsylvanicus and M. montanus males in the patterning of copulatory behavior. It is more likely, however, that the failure is due to differences in the amount of copulatory behavior. M. pennsylvanicus males typically perform approximately 22 intromissions when copulation is allowed to satiety (Gray and Dewsbury, 1974). In comparison, M. montanus males perform approximately 85 intromissions to satiety (Dewsbury, 1973). Female M. montanus require more than two ejaculatory series to insure a high probability of ovulation, but 60% of the females ovulate following two series (Davis et al., 1974). For M. montanus males, these two series include approximately 30 intromissions, an amount similar to the 22 intromissions for M. pennsylvanicus males mated to satiety. It is suggested that these differences in amount of copulatory behavior between M. pennsylvanicus and M. montanus males account for the 50% ovulation percentage. Further research using carefully controlled amounts of copulatory stimulation, either with artificial stimulation or live males, is needed to investigate this failure of maximal ovulation in M. montanus females mated with M. pennsylvanicus males

The lack of a specific vaginal code with respect to patterns of copulatory stimulation in *M. ochrogaster* and *M. montanus* females renders unlikely the hypothesis of reproductive isolation as a function for differences in copulatory patterns among *Microtus*. *M. ochrogaster* females reliably ovulate when mated by males of the sympatric species, *M. pennsylvanicus*. Also, 50% of the *M. montanus* females ovulate when mated by males of a sympatric species, indicating at best an inefficient mechanism of reproductive isolation. It is conceivable that the differential sensitivity of females to specific patterns of copulatory stimulation has been lost as a result of laboratory selection. At present however, the results indicate that the highly stereotyped variations in copulatory pattern among *Microtus* are not related to the stimulation requirements of females.

Inter-specific mating for either *M. ochrogaster* or *M. montanus* females did not result in any implanted embryos. One possible explanation for the failure of implantation is that luteal activity was not induced by the copulatory pattern of *M. pennsylvanicus* males. However, CL in both *M. montanus* and *M. ochrogaster* females mated with *M. pennsylvanicus* males were indistinguishable from functional CL of females mated by conspecific males. A more likely possibility is that inter-specific mating did not result in successful fertilization and development of the ova.

EXPERIMENT 4

It was found in Experiment 3 that inter-specific mating between *M. pennsylvanicus* males and either *M. montanus* or *M. ochrogaster* females did not result in any implanted embryos even though ovulation had occurred. It was suggested that this failure of implantation was due to the absence of fertilization and development of the ova. The following experiment was designed to investigate this possibility.

Methods

Subjects

Subjects were six *M. ochrogaster* females (age 7-10 mos) and five *M. montanus* females (age 4-6 mos). Four *M. pennsylvanicus* males, two *M. montanus* males, and two *M. ochrogaster* males were employed as mating partners. Several additional *M. ochrogaster* and *M. montanus* males were used as stimulus males for the induction of estrus in conspecific females. All animals were of proven fertility.

Procedures

The testing procedure was as described in Experiment 1.

Mating tests for *M. montanus* and *M. ochrogaster* females involved either: 1) copulation with a *M. pennsylvanicus* male, or 2) copulation with a conspecific male. The male and female were observed through one ejaculatory series and then left to copulate for an additional 2 hr; the male was then removed from the cage. Four *M. ochrogaster* females were mated with M. pennsylvanicus males, and two were mated with M. ochrogaster males. Five M. montanus females were mated with M. pennsylvanicus males; of these five, three females evidenced ova in the oviduct and were included in the experiment. Two M. montanus females were mated with M. montanus males.

Females were killed two days following the mating tests. The oviducts were dissected out and examined under a microscope for the presence of ova. Each ovum was examined to ascertain whether it had developed (or cleaved) into the two cell stage or whether it remained undeveloped in the one cell stage.

Results

In all inter-specific matings, the ova were still in the single cell stage and had not undergone cleavage. Ova from females mated with conspecific males had undergone cleavage into the two cell stage.

Discussion

Inter-specific mating between M. pennsylvanicus males and M. montanus and M. ochrogaster females do not result in normal development of the ova. The species thus are not inter-fertile. This lack of inter-fertility constitutes one potential reproductive isolating mechanism for the sympatric species although this does not preclude the possible existence of more efficient, preovulatory isolating mechanisms.

GENERAL DISCUSSION

The present research provides data on the adaptive significance of the elaborateness and diversity of copulatory behavior in three species of *Microtus*. The data relate functions of the behavior to female reproductive physiology. Copulatory behavior plays an essential role in providing sufficient amounts of stimulation to the female for ovulation. Females of all three species require stimulation over and above simple ejaculation for induction of ovulation, and cumulative effects of stimulation on ovulation are found in each species. The specific amount of stimulation necessary for maximal ovulation varies with each species. Indeed, there is an apparent coadaptation of male copulatory behavior and female stimulation requirements with respect to amounts of copulatory stimulation. Both *M. pennsylvanicus* and *M. montanus* females require more than one ejaculatory series for maximal ovulation, and males are capable of achieving a number of series before sexual satiety. In contrast, *M. ochrogaster* females require only a single series for ovulation, and males are rarely capable of more than two series before sexual satiety. It can be concluded that the stimulation requirements of females explain, to a great extent, the elaborateness of copulatory behavior in *Microtus*. They also may explain differences in the pattern of copulation among the species with regard to amounts of copulatory stimulation. This function of copulatory behavior, that of providing sufficient stimulation to the female, may generally account for the elaborateness of the behavior in mammals.

There is no evidence that the stimulation requirements of females explain the highly stereotyped variations among the three *Microtus* species in the temporal and frequency pattern of copulatory behavior. It is also unlikely that the variations in copulatory pattern function as reproductive isolating mechanisms. The results indicate that patterns of copulatory stimulation are not a critical variable in the stimulation requirements of the female. Of course, only two copulatory patterns were compared in *M. ochrogaster* and *M. montanus* females, and a greater range of different patterns may show some selectivity on the part of the females. However, the particular patterns employed were distinct and of clear biological significance since they involved the copulatory pattern of a conspecific male and the copulatory pattern of a male of a sympatric species. Work is in progress in our laboratory on completing all possible inter-specific matings among the three *Microtus* species. Artificial stimulation may also be employed in order to test a wider range of stimulation patterns and to provide better control of specific components of the patterns. The failure of *Microtus* females to demonstrate differential responses to different patterns of copulatory stimulation calls into question the generality of Diamond's concept of vaginal codes. In fact, the only clear support for the concept comes from the study on hamsters. The data for house mice are far from convincing and have been called into question (McGill, 1972). One is left, then, with no clear understanding as to why highly stereotyped species differences in copulatory pattern exist. What is required is substantial research on the relationships of copulatory behavior to a number of factors such as habitat, reproductive anatomy, ecology, social organization, and reproductive physiology.

In addition to providing data on the adaptation of behavior, the investigation of function in behavior involves research on the consequences of behavior, and hence, new data on causal relationships between behavior and other biological phenomena are generated. *Microtus* provide a valuable experimental model for the investigation of the effects of copulatory behavior on ovulation. Inasmuch as they show induced ovulation, the occurrence of ovulation and associated neuroendocrine mechanisms can be dealt with as a discrete event. This is in contrast to almost all other laboratory species which show spontaneous ovulation and short, recurrent estrous cycles. In reproductive systems with spontaneous ovulation, the intricacy of the interplay between ovarian steroids, pituitary gonadotropins, light cycle, and the resulting positive and negative feedback effects makes it difficult to isolate and control discrete events. Research on neuroendocrine mechanisms in *Microtus* should add valuable perspective to our understanding of mammaliam neuroendocrinology.

APPENDIX

TABLE A1

Effects of varying amounts of copulatory stimulation on ovulation and implantation in M. pennsylvanicus females. Results include individual data on number of CL, number of Im. (implanted embryos), Iu. (intra-uterine mortality - CL minus Im.) and on the quantitative measures of copulatory behavior through the first ejaculatory series. Experimental conditions include: 1) Unmated; 2) 1 Ejac. Series (second); 3) 1 Ejac. Series (first); 4) Satiety.

Exp. Condition	Subject	Male	CL	Im.	Iu.
Unmated	1	1	0	0	0
	2	2	0	0	0
	3	3	0	0	0
	4	4	0	0	0
	5	5	0	0	0
	6	6	0	0	0
	7	7	0	0	0
	8	8	0	0	0
	9	9	0	0	0
	10	10	0	0	0
1 Ejac. Series (second)	11	1	0	0	0
	12	2	0	0	0
	13	3	0	0	0
	14	5	0	0	0
	15	6	0	0	0
	16	7	0	0	0

TABLE A1 (extended)

IL	IF-1	TF-1	T/I-1	MF-1	EL-1
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
32	1	16	16.0	2	20
145	4	24	6.0	0	93
292	2	20	10.0	3	41
531	1	18	18.0	0	15
839	1	14	14.0	1	23
206	2	24	12.0	1	35

TABLE A1 (continued)

Exp. Condition	Subject	Male	CL	Im.	Iu.
	17	8	0	0	0
	18	9	0	0	0
	19	10	0	0	0
	20	11	0	0	0
1 Ejac. Series (first)	21	3	0	0	0
	22	6	0	0	0
	23	7	0	0	0
	24	1	5	4	1
	25	2	6	4	2
	26	4	6	5	1
	27	5	3	3	0
	28	10	4	3	1
	29	11	5	4	1
	30	12	3	3	0
Satiety	31	1	7	6	1
	32	2	5	5	0
	33	3	5	5	0
	34	5	6	5	1
	35	6	7	6	1
	36	9	4	4	0
	37	10	5	4	1

TABLE A1 (extended)

IL	IF-1	TF-1	T/I-1	MF-1	EL-1
173	3	24	7.3	2	42
250	1	15	15.0	3	21
430	1	17	17.0	4	23
620	4	24	5.8	3	49
149	16	59	3.7	15	435
337	15	64	4.3	19	531
531	12	52	4.3	24	391
937	21	69	3.3	17	489
209	17	49	2.9	33	631
321	29	84	2.9	41	569
142	13	54	4.2	32	371
730	19	72	3.8	23	621
189	29	94	3.2	25	813
203	16	67	4.2	37	452
601	23	72	3.1	15	416
324	32	94	2.9	18	592
1072	19	70	3.7	28	879
139	15	51	3.4	25	402
1123	14	53	3.8	19	301
208	22	59	2.7	26	376
468	23	73	3.2	45	977

Table A1 (continued)

Exp. Condition	Subject	Male	CL	Im.	Iu.
	38	11	7	5	2
	39	12	7	4	3
	40	13	8	5	3

TABLE A1 (extended)

IL	IF-1	TF-1	T/I-1	MF-1	EL-1
309	29	75	2.6	34	1027
532	19	68	3.6	29	603
794	17	49	2.9	37	423

TABLE A2

Copulatory behavior of *M. pennsylvanicus* males mated with *M. pennsylvanicus* females. Results include individual data for the quantitative measures of copulatory behavior.

	Subjects				
Measure	1	2	3	4	5
IL	601	324	1072	139	1123
IF-1	20	29	17	13	12
TF-1	72	94	70	51	45
T/I-1	3.6	3.2	4.1	3.9	3.6
EL-1	416	592	879	402	301
MF-1	15	18	28	25	19
PEI-1	576	691	783	419	602
IF-2	1	2	1	1	2
TF-2	18	19	15	17	23
T/I-2	18.0	9.5	15.0	17.0	11.5
EL-2	21	39	23	29	42
MF-2	3	1	0	4	3
PEI-2	631	709	810	532	713

TABLE A2 (extended)

Subjects					
6	7	8	9	10	Measure
208	468	309	532	794	IL
19	20	25	17	15	IF-1
59	73	75	68	49	TF-1
3.1	3.7	3.0	4.0	3.3	T/I-1
376	977	1027	603	423	EL-1
26	45	34	29	37	MF-1
438	571	613	498	521	PEI-1
3	2	3	4	1	IF-2
28	20	22	24	14	TF-2
9.3	10.0	7.3	6.0	14.0	T/I-2
59	48	73	27	21	EL-2
5	1	3	2	1	MF-2
553	659	640	597	531	PEI-2

TABLE A2 (continued)

	Subjects				
Measure	1	2	3	4	5
IF-3	2	2	1	3	1
TF-3	17	19	14	21	15
T/I-3	8.5	9.5	14.0	7.0	15.0
EL-3	34	42	21	35	22
MF-3	2	0	1	5	3

TABLE A2 (extended)

Subjects					
6	7	8	9	10	Measure
2	1	1	3	4	IF-3
19	13	15	32	29	TF-3
9.5	13.0	15.0	10.6	7.3	T/I-3
41	19	19	23	39	EL-3
0	4	1	2	3	MF-3

TABLE A3

Copulatory behavior of M. pennsylvanicus males mated with M. ochrogaster females Results include individual data for the quantitative measures of copulatory behavior.

	Subjects				
Measure	1	2	3	4	5
IL	923	814	619	115	139
IF-1	18	13	17	14	27
TF-1	81	70	75	50	76
T/I-1	4.5	5.3	4.4	3.6	2.8
EL-1	432	391	450	210	893
MF-1	18	29	36	49	41
PEI-1	593	601	824	491	732
IF-2	1	1	3	1	4
TF-2	15	13	22	15	29
T/I-2	15.0	13.0	7.3	15.0	7.3
EL-2	32	29	59	22	63
MF-2	4	0	2	0	1
PEI-2	602	1011	829	615	793

TABLE A3 (extended)

Subjects					
6	7	8	9	10	Measure
123	401	213	279	114	IL
18	25	10	15	18	IF-1
63	95	41	53	64	TF-1
3.5	3.8	4.1	3.5	3.6	T/I-1
611	971	302	351	489	EL-1
32	19	27	22	35	MF-1
761	335	409	711	378	PEI-1
2	5	1	2	1	IF-2
21	32	13	14	16	TF-2
10.5	6.4	13.0	7.0	16.0	T/I-2
36	195	29	45	27	EL-2
3	3	4	1	2	MF-2
888	632	517	831	891	PEI-2

TABLE A3 (continued)

	Subjects				
Measure	1	2	3	4	5
IF-3	3	1	2	1	3
TF-3	29	15	21	13	30
T/I-3	9.6	15.0	10.5	13.0	10.0
EL-3	48	21	29	20	48
MF-3	4	0	1	3	3

TABLE A3 (extended)

Subjects					
6	7	8	9	10	Measure
1	1	1	4	2	IF-3
12	13	15	28	19	TF-3
12.0	13.0	15.0	7.0	9.5	T/I-3
21	25	35	72	25	EL-3
3	4	5	1	1	MF-3

TABLE A4

Copulatory behavior of *M. pennsylvanicus* males mated with *M. montanus* females. Results include individual data for the quantitative measures of copulatory behavior.

	Subjects				
Measure	1	2	3	4	5
IL	719	1233	491	822	316
IF-1	14	15	23	26	20
TF-1	52	49	76	71	81
T/I-1	3.7	3.3	3.3	2.7	4.1
EL-1	420	592	608	731	928
MF-1	12	21	32	28	39
PEI-1	601	513	401	619	732
IF-2	4	5	1	2	3
TF-2	26	33	14	21	19
T/I-2	6.5	6.6	14.0	10.5	6.3
EL-2	36	26	15	98	36
MF-2	0	2	0	3	5
PEI-2	631	782	716	694	898

TABLE A4 (extended)

Subjects					
6	7	8	9	10	Measure
915	1032	876	732	918	IL
30	15	13	20	19	IF-1
93	83	48	61	82	TF-1
3.1	5.5	3.7	3.1	4.3	T/I-1
763	1091	532	410	433	EL-1
28	41	36	22	18	MF-1
634	488	572	344	631	PEI-1
3	1	2	1	3	IF-2
25	12	23	16	29	TF-2
8.3	12.0	12.5	16.0	9.6	T/I-2
24	19	21	20	31	EL-2
2	2	3	0	2	MF-2
791	533	681	533	811	PEI-2

TABLE A4 (continued)

	Subjects				
Measure	1	2	3	4	5
IF-3	2	2	1	1	1
TF-3	23	27	14	15	13
T/I-3	11.5	13.5	14.0	15.0	13.0
EL-3	42	36	16	19	17
MF-3	4	2	0	2	3

TABLE A4 (extended)

Subjects					
6	7	8	9	10	Measure
3	1	2	1	3	IF-3
29	13	21	12	23	TF-3
9.6	13.0	10.5	12.0	7.6	T/I-3
29	21	24	12	23	EL-3
0	1	0	1	2	MF-3

TABLE A5

Effect of mating by *M. ochrogaster* and *M. pennsylvanicus* males on ovulation in *M. ochrogaster* females. Results include individual data on the number of CL.

M. ochrogaster male		*M. pennsylvanicus* male	
Subject	CL	Subject	CL
1	3	11	3
2	4	12	5
3	5	13	6
4	5	14	5
5	6	15	4
6	4	16	5
7	3	17	5
8	5	18	3
9	4	19	4
10	6	20	4

TABLE A6

Effect of mating by M. montanus and M. pennsylvanicus males on ovulation in M. montanus females. Results include individual data on the number of CL.

M. montanus male		M. pennsylvanicus	
Subject	CL	Subject	CL
1	7	11	0
2	5	12	0
3	6	13	0
4	7	14	0
5	4	15	0
6	4	16	4
7	5	17	6
8	5	18	5
9	6	19	7
10	7	20	6

REFERENCES

Adler, N. T. (1969). Effects of the male's copulatory behavior on successful pregnancy of the female rat. *J. Comp. Physiol. Psychol. 69*, 613-622.

Aron, C., Asch, G., and Roos, J. (1966). Influence du nombre des coits sur le declenchement de phenomenes de luteinisation ou ovulatoires dan les conditions de las ponte provoquee chez la ratte. *Archiv. Anat. Micro. et Morphol. Exp. 55*, 411-422.

Ball, J. (1934). Demonstration of a quantitative relation between stimulus and response in pseudopregnancy in the rat. *Amer. J. Physiol. 107*, 698-703.

Birdsall, D. A. and Nash, D. (1973). Occurrence of successful multiple insemination of females in natural populations of deer mice (*Peromyscus maniculatus*). *Evolution 27*, 106-110.

Brown-Grant, K., Davidson, J. M., and Greig, F. (1973). Induced ovulation in albino rats exposed to constant light. *J. Endocrinol. 57*, 7-22.

Charlton, H. M., Naftolin, F., and Worth, R. W. (1970). Hypothalamic influence on pituitary and serum levels of LH in the vole, *Microtus agrestis*. *Proc. Physiol. Soc.*, 7-8.

Chester, R. V. and Zucker, I. (1970). Influence of male copulatory behavior on sperm transport, pregnancy, and pseudopregnancy in female rats. *Physiol. Behav. 5*, 35-43.

Christian, J. J. and Davis, D. W. (1964). Endocrines, behavior, and population. *Science 146*, 1550-1563.

Clulow, F. V. and Mallory, F. F. (1970). Oestrus and induced ovulation in the meadow vole, *Microtus pennsylvanicus*. *J. Reprod. Fert. 23*, 341-343.

Conaway, C. H. (1971). Ecological adaptation and mammalian reproduction. *Biol. Reprod. 1*, 239-247.

Davis, H. N., Gray, G. D., Zerylnick, M., and Dewsbury, D. A. (1974). Ovulation and implantation in montane voles (*Microtus montanus*) as a function of varying amounts of copulatory stimulation. *Horm. Behav.*, In press.

Dempsey, E. W. and Searles, H. F. (1943). Environmental modification of certain endocrine phenomena. Endocrinology 32, 119-128.

Dewsbury, D. A. (1970). Copulatory behavior of rice rats (Oryzomys palustris). Anim. Behav. 18, 266-275.

Dewsbury, D. A. (1971). Copulatory behavior of old-field mice (Peromyscus polionotus subgriseus). Anim. Behav. 19, 192-209.

Dewsbury, D. A. (1972) Patterns of copulatory behavior in male mammals. Quart. Rev. Biol. 47, 1-33.

Dewsbury, D. A. (1973). Copulatory behavior of montane voles (Microtus montanus). Behaviour 44, 186-202.

Dewsbury, D. A. (1974). Copulatory behaviour of white-throated woodrats (Neotoma albiguli) and golden mice (Ochrotomys nuttalli). Anim. Behav. 22, 601-610.

Dewsbury, D. A. and Estep, D. Q. (1974). Copulatory behavior of Peromyscus eremicus: effects on the initiation of pregnancy. Paper presented at Animal Behavior Society, University of Illinois, Urbana, Illinois, May.

Dewsbury, D. A. and Jansen, P. E. (1972). Copulatory behavior of southern grasshopper mice (Onychomys torridus). J. Mammal. 53, 276-278.

Diamond, M. (1970). Intromission pattern and species vaginal code in relation to induction of pseudopregnancy. Science 169, 995-997.

Diamond, M. (1972). Vaginal stimulation and progesterone in relation to pregnancy and parturition. Biol. Reprod. 6, 281-287.

Diamond, M. and Yanagimachi, R. (1968). Induction of pseudopregnancy in the golden hamster. J. Reprod. Fert. 17, 165-168.

Dilley, W. G. and Adler, N. T. (1968). Post-copulatory mammary gland secretion in rats. Proc. Soc. Exp. Med. 129, 964-966.

Duffy-Barbe, L., Franchimont, P., and Faure, J. M. A. (1973). Time-courses of LH and FSH release after mating in the female rabbit. Endocrinology 92, 1318-1321.

Ericsson, R. J. and Baker, V. F. (1966). Induced ovulation in female rats exposed to neonatal androgen. Proc. Soc. Exp. Biol. Med. 122, 88-92.

Everett, J. W. (1939). Spontaneous persistant estrus in a strain of albino rats. Endocrinology 25, 123-127.

Fernandez-Baca, S., Madden, D. H. L., Novoa, D. (1970). Effect of different mating stimuli on induction of ovulation in the alpaca. J. Reprod. Fert. 22, 261.

Gray, A. P. (1970). Mammalian Hybrids. Commonwealth Agricultural Bureaux, Farnham Royal, England.

Gray, G. D., Davis, H. N., Zerylnick, M., and Dewsbury, D. A. (1974). Oestrus and induced ovulation in montane voles. J. Reprod. Fert. 38, 193-196.

Gray, G. D. and Dewsbury, D. A. (1973). A quantitative description of copulatory behavior in prairie voles (Microtus ochrogaster). Brain Behav. Evol. 8, 437-452.

Gray, G. D. and Dewsbury, D. A. (1974). A quantitative description of the copulatory behaviour of meadow voles (Microtus pennsylvanicus). Anim. Behav., In press.

Gray, G. D., Zerylnick, M., Davis, H. N., and Dewsbury, D. A. (1974). Effects of variations in male copulatory behavior on ovulation and implantation in prairie voles, Microtus ochrogaster. Horm. Behav., In press.

Hall, E. R. and Kelson, K. R. (1959). The Mammals of North America. Ronald Press, New York, Vol. II.

Jöchle, W. (1973). Coitus-induced ovulation. Contraception 7, 523-564.

Koplin, J. R. and Hoffmann, R. S. (1968). Habitat overlap and competitive exclusion in voles (Microtus). Amer. Midl. Nat. 80, 494-507.

Krebs, C. J., Keller, B. L., and Tamarin, R. H. (1969). Microtus population biology: demographic changes in fluctuating populations of M. ochrogaster and M. pennsylvanicus in southern Indiana. Ecology 50, 587-607.

Lanier, D. L., Estep, D. Q., and Dewsbury, D. A. (1974). Induction of pregnancy in the Syrian golden hamster. Paper presented at Eastern Regional Conference on Reproductive Behavior, Emory University, Atlanta, Georgia, June.

Lee, C. and Horvath, D. J. (1969). Management of the meadow vole (Microtus pennsylvanicus). Lab. Anim. Care 19, 88-91.

Lee, C., Horvath, D. J., Metcalfe, R. W., and Inskeep, E. K. (1970). Ovulation in Microtus pennsylvanicus in a laboratory environment. Lab. Anim. Care 20, 1098-1102.

McGill, T. E. (1972). Preejaculatory stimulation does not induce luteal activity in the mouse Mus musculus. Horm. Behav. 3, 83-85.

McGill, T. E. and Coughlin, R. C. (1970). Ejaculatory reflex and luteal activity in Mus musculus. J. Reprod. Fert. 21, 215-220.

Miller, W. C. (1969). Ecological and ethological isolating mechanisms between Microtus pennsylvanicus and Microtus ochrogaster at Terre Haute, Indiana. Amer. Midl. Nat. 82, 140-148.

Moss, R. K. and Cooper, K. J. (1973). Temporal relationships of spontaneous and coitus-induced release of luteinizing hormone in the normal cycling rat. Endocrinology 92, 1748-1753.

Pearson, O. P. (1944). Reproduction in the shrew (Blarina brevicauda Say). Amer. J. Anat. 74, 39-93.

Richmond, M. and Conaway, C. H. (1969a). Induced ovulation and oestrus in Microtus ochrogaster. J. Reprod. Fert., Suppl. 6, 357-376.

Richmond, M. and Conaway, C. H. (1969b). Management, breeding, and reproductive performance of the vole, Microtus ochrogaster, in a laboratory colony. Lab. Anim. Care 19, 80-87.

Rodgers, C. H. (1971). Influence of copulation on ovulation in the cycling rat. Endocrinology 88, 433-436

Seidel, D. R. and Booth, E. S. (1960). Biology and breeding habits of the meadow mouse, Microtus montanus, in eastern Washington. Walla Walla Coll. Publs. Depart. Biol. Sci. 29, 1.

Sighoret, J. P., Du Mesnil du Buisson, F., and Mauleon, P. (1972). Effect of mating on the onset and duration of ovulation in the sow. J. Reprod. Fert. 31, 327-329.

Sullivan, J. J., Bartlett, D. E., Elliot, F. I., Brouwer, J. R., and Kloch, F. B. (1972). A comparison of recto-vaginal, vaginal, and speculum approaches for insemination of cows and heifers. A. I. Digest 20, 6.

Van der Westhuysen, J. M., van Niekerk, C. H., and Hunter, G. L. (1970). Duration of oestrus and time of ovulation in sheep: effect of synchronization, season, and ram. Agroanimalia 2, 131.

Wilson, J. R., Adler, N. T., and LeBoeuf, B. (1966). The effects of intromission frequency on successful pregnancy in the female rat. Proc. Nat. Acad. Sci. 53, 1392-1395.

Zarrow, M. X., Campbell, P. S., and Clark, J. H. (1968). Pregnancy following coital-induced ovulation in a spontaneous ovulator. Science 159, 329-332.

Zarrow, M. X. and Clark, J. H. (1968). Ovulation following vaginal stimulation in a spontaneous ovulator and its implications. J. Endocrinol. 40, 343-351.

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